

### Coagulation tests at ambient temperature

The present invention relates to a method of determining prothrombin time (PT) in a whole blood, anti-coagulated blood, blood plasma or anti-coagulated blood plasma sample at ambient temperature and to a test kit for performing an analysis according to said method.

#### **Background of the invention**

Prothrombin time (PT) is a laboratory diagnostic coagulation test by which the functionality of the extrinsic coagulation pathway is assessed. Coagulation tests are defined as test performed on biological fluids such as blood, anti-coagulated blood, blood plasma or anti-coagulated blood plasma, for which tests the end-point is a coagulation phenomena, such as formation of a gel or a clot or the dissolution of gel or clot. In more modern terminology, the end-point of a coagulation test involves the formation of fibrin from fibrinogen or the dissolution of fibrin into fibrin degradation products.

Prothrombin time (PT) tests are used to diagnose liver disease and coagulation anomalies of the blood. PT tests are also used to monitor antithrombotic therapy by vitamin K antagonists. In performing a PT test, sample and liquid thromboplastin reagent are mixed at 37°C and the time needed for the mixture to clot is determined. When the test was first devised more than fifty years ago, this clotting time was identical to the PT, but today, to accommodate variations in test procedure properties, the PT is expressed in International Normalized Ratio (INR), see below.

PT tests measure the time from exposure to membrane bound tissue factor (thromboplastin) to the detection of a clot. Two types of PT tests are recognized, Quick PT and Owren PT, named after the first author as they were first described in the literature, Quick A et al. The prothrombin time in hemophilia and in obstructive jaundice. J Biol Chem 1935; 109: 73-4 and Owren P and Aas K. The control of dicumarol therapy and quantitative determination of prothrombin and proconvertin. Scand J Clin Lab Invest 1951; 1951; 201-8. Both the Owren PT and the Quick PT are wet-chemistry coagulation tests, i.e. the sample is mixed with liquid (not dry) reagent. The difference in the procedures resides in the fact that the Quick PT reagent contains only thromboplastin whereas the Owren PT reagent also contains fibrinogen and coagulation factor V. The fibrinogen content of the reagent may be viewed as discriminatory. If the PT reagent contains sufficient amounts of fibrinogen to increase the fibrinogen content of the mixture of sample and reagent by at least 0.1 g/L then it is an Owren-type PT test, not a Quick PT. This is even more true when the increase is at least 0.5 g/L. Another cardinal distinguishing feature is that the relationship between reagent and sample is greater than four, typically greater than ten, in a Owren-type PT procedure whereas it is hardly ever greater than two in the Quick-type. The publication Lindahl et al.

Plasma-based calibration of Owren-type prothrombin time with results expressed in INR. Thromb Haemost 2004; 91: 1223-31, submitted to publication to several journals during the past six years, was published 8 months after the filing of the priority application of the present invention, but may be viewed as containing relevant background data.

5 Today, the result of a PT test is not expressed in clotting time (CT) but rather in International Normalized Ratio (INR). The INR is obtained by comparing the CT for the sample with a sample of an average normal individual, normal clotting time ((NCT). The INR is the ratio between CT and NCT raised to the power of ISI, the International Sensitivity Index. See the above given references of van den Besselaar et al and Pollar et al. The NCT 10 and ISI are properties of the certain PT test procedure and are obtained by calibrating the test. In the calibration of a given PT test procedure the results are directly or indirectly aligned with those of a reference PT test procedure.

15 According to prior art, laboratory diagnostic coagulation tests, such as PT, are performed at 37°C to mimic the physiological situation. The difficulties in standardizing a PT test at this temperature, or at any temperature, are considerable as is evident from on-going international work in the area; van den Besselaar AM. International normalized ratio: towards improved accuracy. Thromb Haemost 1999; 82: 1562-3, and Pollar L et al Concerted Action 20 on Anticoagulation (ECAA) – the multicentre calibration of rabbit and human ECAA reference thromboplastins. Steering Committee. Thromb Haemost 1996; 76: 977-82. However, the lock-in of PT at 37°C presents a hurdle in design of test procedures, especially for those intended to be used close to the patient, i.e. in point of care (POC) PT testing. To be able to maintain a temperature of 37°C during the test performance, a thermostat set at 37°C is 25 needed, and a temperature-equilibration time must be allowed. The need of a thermostat adds much hardware and costs to the currently available instrumentation and hampers the development toward increased point of care (POC) testing. The notion to perform a PT test at ambient temperature, especially at room temperature, is very attractive.

30 Many PT tests are performed on blood plasma at highly automated centralized laboratories. Although this may be economical for generating the test results, it may not be overall economical from a patient-care point of view. The reason is the time required to transport samples to the laboratory and to get the results back. This time can be hours to days. This is long time compared to the 15 minutes or less that would be required for a PT test to be performed next to the patient, that is at the point of care (POC). The transportation time can delay the start of optimal treatment and can force the patient to make additional 35 visits to the physician. In addition, a centralized PT analysis will require a vein puncture, a relatively large blood sample volume and a sturdy blood container. A POC PT analysis only requires a fingerstick, a small blood volume and no sample container for transportation of the sample. Considerations of this kind have prompted the development of POC PT test

procedures intended to be performed at doctors' offices, at small laboratories and at patients' homes.

As alluded to above, the PT test represents a considerable standardization challenge which calls for caution in moving away from established test conditions. A tempting move in the design of POC PT test procedures is the move from established wet chemistry to dry chemistry because the latter is more readily adapted to the demands of a POC laboratory test situation. Inventive aspects of dry chemistry POC PT test design are found in US patent 6,402,704B1 to McMorrow, US patent 6,103,196 to Yassinzadeh et al, US patent 5,302,348 to Cusack et al, US patent 4,849,340 to Oberhardt and the International patent application 10 WO 93/22453 of Zweig. The latter of these describes a test article for determining coagulation capability in a blood sample comprising a porous membrane having coagulation initiator and substrate impregnated therein. The test is not a coagulation test since the end-point is not a detected clotting, what is detected is the cleavage of a synthetic substrate by some enzyme, possibly thrombin. In the description of the invention, it is proposed that the 15 test response could, by mathematical means, be made equivalent to the PT of the sample also when the test is performed at ambient room temperature. No quantitative proof of this is presented.

There are about 4 million European patients on vitamin K antagonist therapy who need to have their PT determined about 20 times per year. This makes for 80 million PT tests 20 per year, each of which requires about 15 minutes of health care time and contributes with about 10 Euro per test in health care costs, not counting test materials which add about 0.5 Euro per test. The health care cost of PT testing in monitoring antithrombotic therapy, not counting test materials, is thus about 800 million Euro. If these health care costs could be reduced by 20% by increased frequency of home POC PT testing, while enhance the quality 25 of life for the patient, health care savings of 160 million Euro would result. Even after an offset by about 2 Euro per test in increased material costs, the savings in the health care sector would be about 130 million Euro.

### Description of the invention

30 The present invention is in one aspect directed to a method of determining prothrombin time (PT) in a whole blood, anti-coagulated blood, blood plasma or anti-coagulated blood plasma sample. The method comprises the steps of  
a) adding a defined volume of the sample to a defined volume of a liquid reagent, or vice versa, in a vessel,  
35 b) determining the temperature of the reaction mixture of a) at an ambient temperature in the range of 15°C to 45°C,  
the steps a) and b) being performed in an optional order, followed by

c) determining a clotting time (CT) by registering the time from the addition in a) until a clot is detected in the vessel,

and

5 d) calculating the prothrombin time (PT) based on the temperature of b) and the clotting time (CT) of c).

It should be understood that the use of the term "vessel" comprises all kinds of containers for liquids, such as test tubes, cuvettes, vials etc.

In an embodiment of the invention, the liquid reagent comprises two or three different reagents.

10 In another embodiment the liquid reagent contains an amount of fibrinogen increasing the concentration of fibrinogen in the mixture of a) by at least 0.1 g/L and the ratio between the defined volume of the reagent and sample in a) is at least four. In this other embodiment of the invention, the PT test procedure may be even more pronounced an Owren-type PT procedure in that the liquid reagent contains an amount of fibrinogen  
15 increasing the concentration of fibrinogen in the mixture of a) by at least 0.5 g/L and the ratio between the defined volume of the reagent and sample in a) is at least ten.

In a preferred embodiment of the invention the calculated prothrombin time (PT) is expressed as International Normalized Ratio (INR).

20 In a variant of the method of the invention, the determination of the temperature in step b) is accomplished by determining the ambient room temperature, provided that the liquid reagent is of the same temperature.

25 As mentioned, the method of the invention may be performed at an ambient temperature of 15°C to 45°C, but a more preferred temperature range is 18°C to 35°C. A most preferred temperature range under the conditions used in the examples is 30°C to 35°C.

In yet another preferred embodiment of the invention the International Normalized Ratio (INR) of the sample is calculated using the equation

$$INR = (CT/(NCT(t)))^{ISI(t)}$$

30

wherein

CT = the clotting time of e) in claim 1,

t = the temperature of c) in claim 1,

NCT(t) = Normal Clotting Time (NCT) expressed as a function of the temperature t, and

35 ISI(t) = International Sensitivity Index (ISI) expressed as a function of the temperature t.

In still another embodiment of the invention the prothrombin time (PT) is obtained from a table with rows and columns where one is for various clotting times, and the other is

various temperatures, and the PT is found in the intersection of clotting time and temperature.

Another aspect of the invention is directed to a test kit for performing an analysis according to the method aspect of the invention that comprises temperature recording means, 5 and one or several separate sealed vessels containing reagents for clotting one or more defined volumes whole blood, anti-coagulated blood, blood plasma or anti-coagulated blood plasma sample.

Also in this second aspect of the invention the term "vessel" should be interpreted broadly to comprise all kinds of containers for liquids, such as test tubes, cuvettes, vials etc.

10 The temperature recording means can be any means with which the ambient temperature in the range of recording 15°C to 45°C can be recorded, such as an ordinary glass thermometer, a thermistor etc. The thermometer may also be an especially designed thermometer that has a hook at one end, thus enabling visual detection of clot formation in the vessel as the thermometer is moved to and fro.

15 In an embodiment of the invention a reagent in a vessel contains fibrinogen in an amount that yields a final fibrinogen concentration of at least 0.1 g/L in a vessel when mixed with a sample to be tested.

In a preferred embodiment the reagents in the vessels are in lyophilized form for reconstitution with one or more defined volumes of liquid prior to use.

20 Even though it is not necessary to provide time registration means, such as a stopwatch or electronic timer with a test kit, it will be convenient for the end user to have all necessary equipment in one test kit. Therefore, it will also be suitable to include volume determining means, such as pipettes or the like into the test kit.

25 Calibration of a PT test involves the whole procedure or method. It involves the reagent(s), the instrument, the volumetric equipment and the instructions for use. Since many users, especially POC PT tests users, want to rely on the calibration of the manufacturer, it follows, that the calibration of a manufacturer will be reliable only if the product delivered, the equipment kit, contains everything needed to perform the PT test. However, according to prior art, this is not the case. Because of the high costs of the 30 instrument, the instrument will be delivered separately and will be used throughout an extended time period, e.g. three to five years. The instrument will not be replaced every time a new lot of reagent is taken into use, which may be about every 6 to 12 months. However, the present invention will be useful in the development of a cheap instrument that can be disposed of after use of a reagent lot comprised by a test kit.

35 The main reason to perform a coagulation tests at ambient temperature is to abolish the need of expensive and energy consuming temperature control equipment. According to the present invention, it is possible to perform coagulation tests at the ambient temperatures

typical of human dwellings. According to the present invention, it is possible to perform a coagulation test at ambient temperature in which the clotting time and the temperature are measured and used to obtain the test results. In performing a coagulation test according to the invention, there is also a second reason to measure the temperature. This is to qualify 5 the temperature conditions of the test.

The test results of a PT test is expressed in INR. According to prior art, a PT test is performed at a fixed temperature of 37°C and the INR is a function of the clotting time, INR(CT). As opposed to this, a PT test is performed according to the invention at any 10 temperature within the specified limits. The clotting time and the temperature (t) are both measured and the INR is a function of both, INR(CT, t). The temperature is also measured to ascertain that the temperature is within a range where the PT test according to the 15 invention may be performed.

The coagulation test is intended to be performed at temperatures typical of human 20 dwellings. Hence, the ambient temperature range is assumed to be the indoor temperature range of such a dwellings, i.e. between 15°C and 45°C. It is assumed within the scope of the 25 present invention that the ambient temperature is limited to the range of 15°C and 45°C.

A sharp dependency of the clotting time with temperature is likely to reduce the 20 precision of the assay. As evidenced in Example 1, the speed of clot formation is reduced at temperatures below 18°C making clotting less distinct and more difficult to detect. Also at 25 temperatures above 35°C the PT results were dependent on temperature. It is therefore preferred in the present invention to perform PT test by a procedure at an ambient temperature in the range of 18°C to 35°C.

In antithrombotic treatment with vitamin K antagonists the optimal blood PT level is 25 in the range of INR 2 to INR 3. The results of experimental work described in Example 1 indicate that there is an optimal temperature range for determination of PT in samples with INR above 2, see Figure 3. This optimal temperature range is about 30°C to 35°C. In 30 performing coagulation tests according to the invention, it may be favorable to so in the ambient temperature range of 30°C to 35°C.

Without departing from the spirit of the invention, other temperature limits than those 30 mentioned above are possible. As indicated, the lower limit of the range of permissible temperatures appears to be the most critical and may needed to be fine tuned, e.g. specified to 22°C. Although, no reason has appeared why the upper limit of permissible temperatures should be lower than 35°C, such reason could well appear. Reduction of the upper limit, e.g. to 30°C, is possible within the scope of the present invention.

35 As mentioned above, there appears to be an optimal temperature range for determination of PT in samples with INR in the range 2 to 3 or even the range of 2 to 7. In

this optimal temperature range, the temperature dependence of the test result is small which will minimize the impact of bad temperature equilibration and/or determination. In the examples below, this optimal temperature range was about 30°C to 35°C. The important observation is that there is an optimal temperature range and it is conceived that alterations 5 in reagent composition and/or procedural details can shift this optimal temperature range to lower temperatures including those typically found in human dwellings, i.e. 20°C to 30°C. If such a shift in optimal temperature can be accomplished, then there are reasons to adjust the limits of permissible temperature accordingly.

The invention also concerns how to obtain results from PT tests performed 10 according to the invention. In a PT procedure according to the invention, the clotting time and the temperature are determined. As shown in Figure 1 and Figure 2, the NCT and the ISI for a given PT procedure can be viewed as functions of temperature and the nature of these functions may be determined by calibration. The PT procedure according to the invention can be calibrated by determining the clotting time at various temperatures within the permissible 15 range for samples with known INR. At these temperatures, the NCT and the ISI of the PT procedure are determined and functional relationships between normal NCT and temperature and ISI and temperature. The later are denoted NCT(t) and ISI(t), respectively. Once, NCT(t) and ISI(t) are determined, the INR as function of clotting time (CT) and temperature, INR(CT, t) can be determined as follows:

20

$$\text{INR}(\text{CT}, t) = (\text{CT}/\text{NCT}(t))^{\text{ISI}(t)}$$

In the examples, NCT(t) and ISI(t) were second degree polynomials. Naturally many other types of functions may find use in this context. Any function that gives a good fit to the 25 calibration data may be used.

As an alternative to deducing a functional relationship between INR and t and CT, i.e. deducing a two variable expression NCT(t, CT) and computing the assay results using this expression, a table may be constructed. This table will be a matrix where each row is a clotting time (CT) and each column a temperature. Each given pair of temperature and CT 30 will correspond to an INR. Thus, there is no departing from the spirit of the invention if the results of the PT procedure is presented in a table where the results of the test is in position defined by a temperature and a clotting time.

Without departing from the scope of the invention, any method that allows an INR 35 value to be deduced from a CT and a temperature may be employed. The same is true for assay conditions. These may include any limited permissible temperature range and any limited permissible clotting time range. In the experimental work there are temperature ranges identified for which the temperature dependence is negligible and which makes the

INR value a function of the clotting time alone. Also in this case the PT test is according to the invention. The clotting time and the temperature are both measured and used to obtain the test results. The temperature is measured to ascertain that the temperature is within a permissible range.

5 It is a part of the invention to provide users equipment needed to perform a PT procedure according to the invention. The test kit of the invention may, in addition to the components already mentioned contain an instrument and instructions for use. A test kit may e.g. contain reagents for 10 to 100 PT analysis.

10 **Description of the drawings**

Fig.1 is a diagram wherein the filled circles show the NCT of Table 1. The solid line is second-degree polynomial fitted to the experimental data points by minimal least square method. The open circles show the NCT values for the PT procedure estimated with the patient plasma samples and the INR values for these supplied by the department. The fitted function was  $NCT(t) = 136.4 - 6.165t + 0.0837t^2$ .

15 Fig. 2 is a diagram wherein the filled circles show the experimentally determined ISI data of Table 1. The solid line is second-degree polynomial fitted to the experimental data points by minimal least square method. The open circles show the NCT values for the PT procedure estimated with the patient plasma samples and the INR values for these supplied by the department. The fitted function was  $ISI(t) = 1.0584 + 0.04201t - 0.0011t^2$ .

20 Fig. 3 is a diagram wherein the solid lines show the expected clotting time with the PT procedure for samples with INR values of 1, 2, 3, 4, 5 and 7 in the temperature range of 18°C to 40°C. The calculations were made with the relationship  $INR = (CT/NCT)^{ISI}$ , rearranged into  $INR = (CT/NCT(t))^{ISI(t)}$ , with insertions of the  $NCT(t)$  and  $ISI(t)$  given in legends to Figures 1 and 2.

25 Fig. 4 is a diagram wherein INR values of 23 patient citrated plasma samples determined according to the invention at 22.7°C are compared with INR values for the same samples determined according to prior art. by the Department of Clinical Chemistry at the University Hospital, Linköping, Sweden.

30 Fig. 5 is a diagram wherein the average INR value of three determinations according to the invention for 12 patient plasma samples, at 18.5°C, 22.7°C and 28.4°C, were compared to the INR values of the same samples determined according to prior art. The determinations according to the invention and according to prior art are denoted INR invention and INR dept clin chem, respectively.

## EXAMPLES

The following examples are given to a better understanding of the invention and to show that the invention is of practical and economic importance. The examples show but a trifle of all possible embodiments of the invention. Therefore, the examples do not limit in any 5 way the scope and spirit of the present invention.

### Materials and Methods.

PT reagent was GHI 131 lot C225F which is of the Owren's kind in that it contains rabbit brain thromboplastin and bovine factor V and bovine fibrinogen. The reagent, supplied as a lyophilized powder. One vial of reagent was reconstituted in first 5 mL of water and then 10 5 mL of 25 mM CaCl<sub>2</sub>. The reagent was used within 4 to 10 hours of reconstitution. In the PT test procedure, 300 µL of reagent was placed in the reaction vessel to which 25 µL of sample was added, a ratio of 12. The amount of fibrinogen in the reagent was sufficient to increase the fibrinogen concentration in the mixture of sample and reagent by about 0.8 g/L.

Control plasma samples with known INR were lyophilized plasma samples with 15 known INR. One control plasma, product number GHI 162, had an INR of 1.14 and the other, product number GHI 167, had an INR of 2.82. The lyophilized control plasmas were first reconstituted in 1 mL of water according to the manufacturers recommendation, and then, to give the reconstituted control plasma about the same PT potency as blood with erythrocyte fraction volume (EVF) of 0.45, the control plasma was diluted by addition of 0.82 mL of 150 20 mM NaCl. The PT reagent and control plasmas were manufactured by Global Hemostasis Institutet MGR AB, Linköping, Sweden.

Citrated plasma samples were from the Department of Clinical Chemistry, University Hospital, Linköping, Sweden. The 23 samples were from those arriving to the department for analysis of PT during about 3 hours in the afternoon of June 12, 2003. The samples were 25 analyzed according to the invention within 4 hours of being analyzed by the routine procedure of the department. The samples were made untraceable to preserve the anonymity of the patients and were provided with the PT values obtained by the department. Prior to assay according to the invention, a 200 µL portion of each plasma was diluted 1:1.82 by addition of 164 µL of 150 mM NaCl. This was to make them equipotent with blood with 30 EVF of 0.45. The clinical experiments were conducted in collaboration with Professor Tomas L. Lindahl, Department of Clinical Chemistry, University of Linköping and approved by the local ethical committee of the Medical Faculty of the University of Linköping.

Glass capillaries with a volume of 25 µL (length 50 mm), product number 111.395-25, were from KEBO-lab, Stockholm., Sweden.

35 The PT reagent was added to 8 mm inner diameter polystyrene tubes, 300 µL of reagent in each. The tubes were capped and temperature equilibrated in a water bath. At

time zero, 25  $\mu$ L of sample was added to a reagent tube and recapped. The tube was tilted once about every second so that the bottom of the tube was out of water bath when tilted and back in the water bath when straight up. The contents were inspected every time the tube was tilted. When first signs of clotting were detected the time was recorded. The 5 temperature in the water bath was varied between 14°C and 40°C. The 25  $\mu$ L of sample was added either by glass capillary or by pipette with disposable plastic tip.

**Example 1.**

With a PT procedure, the clotting time (CT) was determined for two control plasma samples at every two degrees centigrade in the temperature range of 18°C to 38°C. The 10 control plasma samples had INR values of 1.14 and 2.82. From these values, the Normal Clotting Time (NCT) and International Sensitivity Index (ISI) of the PT procedure were calculated at each of the eleven temperatures. The NCT was in the range of 55 sec to 22 sec and the ISI was in the range 1.0 to 1.5, See Figures 1 and 2. Second-degree polynomials were fitted by minimal least square method to give NCT and ISI as functions of temperature. 15 The second-degree polynomials that gave the best fit to the data were:

$$NCT(t) = 136.4 - 6.165t + 0.0837t^2$$

$$ISI(t) = 1.0584 + 0.0420t - 0.0011t^2$$

NCT and ISI for the same PT procedure was also determined at three temperatures, 18.5°C, 22.7°C and 28.4°C with patient plasma samples from the Department of Clinical 20 Chemistry. Figure 1 and 2 also show that the estimates of NCT and ISI made with the patient plasma samples and the INR values supplied by the department were well in line.

The functional relationship between the temperature and NCT and ISI allowed the construction of schools of curves, each for a specific INR value, showing the expected clotting time as function of temperature. The curve for INR 1 flattened out in the temperature 25 range of 35°C to 40°C. The curves for higher INR values displayed an increasingly pronounced minimum in the range 30°C to 35°C.

Example 1 demonstrates that functional relationships of temperature and NCT and ISI,  $NCT(t)$  and  $ISI(t)$ , can be determined. Furthermore, Example 1 demonstrates that every value pair  $(t, CT)$  translates into an INR value. Figure 3 could be used for such a translation 30 but an interpolation between the curves is necessary. A two variable mathematical relationship or a table appears to be the most straight forward means to translate the  $(t, CT)$  value pair into an INR value. Figure 3 clearly demonstrates that a translation of this kind can be performed in practice.

When a sample is analyzed according to the invention, the clotting time and the 35 ambient temperature ( $t$ ) are measured. With these values and known functional relationships

NCT (t) and ISI(t), an INR value can be computed as  $INR = (CT/NCT(t))^{ISI(t)}$ . Thus, according to the invention, provided the temperature is within a permissible range, every value pair (t, CT) will generate an INR value. Naturally, the CT must also be within some permissible range but this does not represent anything new in comparison to state of art PT procedures

5 and is therefore not elaborated on here.

**Example 2.**

Plasma samples from 23 patients were obtained from the Department of Clinical Chemistry together with INR values determined according to prior art. The 23 samples were analyzed according to the invention. The ambient temperature and the clotting time for each

10 sample were measured. The temperature in the series was stable at 22.7°C. At this temperature, the NCT and ISI of the PT procedure was determined with respect to the prior art INR values. INR values according to the invention were then computed. The INR values obtained according to the invention at 22.7°C were compared to the INR values obtained according to prior art at 37°C, see Figure 4. The comparison was by linear regression. A

15 slope of near unity (1.008), an intercept close to zero (0.007) and a correlation coefficient of 0.982 was obtained. There were no obvious outliers. The first 12 of the 23 samples were similarly analyzed at 18.5°C and 28.4°C. For each of these 12 samples a mean INR value was determined as the average of three determinations, one at each of the three mentioned temperatures. This mean INR value was also compared by linear regression analysis to the

20 INR values supplied by the department, Figure 5. Interestingly, the mean INR values correlate more strongly with the INR values supplied by the department than the INR values obtained at only one temperature (22.7°C), squared regression coefficient was 0.995 compared to 0.982. Inspection of the data in Figure 5, shows no bias and no obvious outliers. It appears that INR can be determined according to the invention at any temperature in the

25 range of 18°C to 40°C.

Example 2 demonstrates that INR values can be determined at other temperatures than 37°C. According to the data, a PT test can be performed at ambient temperature provided that the clotting time and the temperature are measured and that both of these values are used to derive the test result. To further prove the point, mean INR values were

30 determined for 12 patient plasma samples. Each mean INR value was an arithmetic average of three INR determinations, one at 18.5°C, a second at 22.7°C and a third at 28.4°C. The mean INR values and the prior art INR values were compared in Figure 5. In this case the data lined up even better than in Figure 4. The results are interpreted in the following way; each mean INR value is the average three determinations according to the invention. Each of

35 these three estimates is equally good, regardless of temperature. The mean INR values are therefore better estimates of the true INR value than the single estimate at 22.7°C. A

standard deviation of the determinations are obtained by assuming that the INR value of the department was correct. The standard deviation for the mean INR and the 22.7°C INR can then be determined to 0,029 and 0.043, respectively. The ratio between these standard deviations is 1.5 which is close to 1.73, the square root of three, which is expected if all 5 determinations, regardless of temperature, carry the same weight.

The data shown in Figures 4 and 5 display a discovery on which the invention is based. PT can be determined at any temperature in the range of about 15°C to 45°C. The temperature needs only be measured and used together with the clotting time to obtain the assay result. The fact that PT can be determined over a relatively broad temperature range is 10 not trivial. It is a fact that now has been experimentally verified. Now we know, that new analytical possibilities, offering considerable advantages, open themselves unto us. Since the temperature does not by necessity need to be defined, it only needs to be measured, much expensive hardware can be stripped from the instrument used for PT determination. It could well be that the instruments can perhaps be made so inexpensive that a disposable 15 instrument for PT tests is possible. For POC PT testing this could be decisive.

It is likely that the invention may be practiced on any type of wet-chemistry PT test. In particular is clearly demonstrated that the invention may be practiced with a wet-chemistry PT test of the Owren type, i.e. with PT test in which the reagent contains sufficient amounts of fibrinogen to increase the fibrinogen concentration in the mixture of at least in the range of 20 0.1 g/L to 0.5 g/L and in which the volumetric ratio reagent to sample is at or above the range of four to ten. The volumetric ratio may, however, be much higher, e.g. 50 or even higher.

PT determination according to the invention could result in improved test quality. One reason for a bad PT result is temperature fault. The temperature in the reaction mixture is not what it is assumed to be. This could be because of the temperature has not had the 25 time to equilibrate, or because of some error in the thermostat. It stands clear to reason that it is easier to measure a temperature with good precision than to keep it at some predetermined level with the same good precision.

Table 1. The PT clotting time for the two control plasma samples were determined at various temperatures in the range of 18°C to 38°C. The clotting times are designated CT INR 1.14 and CT INR 2.82. Using these two CT values, the NCT and the ISI of the PT procedure was determined at each temperature from the relationship  $INR = (CT/NCT)^{ISI}$ . All clotting times are given in seconds.

	Temperature	CT INR 1.14	CT INR 2.82	NCT	ISI
10	18°C	57	109	51.9	1.40
	20°C	52	97	47.5	1.45
	22°C	45	83	41.2	1.48
	24°C	40	75	36.5	1.44
	26°C	35	68	31.8	1.36
	28°C	33	63	30.1	1.40
	30°C	30	61	27.1	1.28
	32°C	27	60	24.1	1.13
	34°C	26	57	23.2	1.15
	36°C	26	57	23.2	1.15
15	38°C	26	63	22.9	1.02

20

25

30

35

**Table 2.** Below is a piece of an Excel file showing that a clotting time and a temperature, measured when practicing a PT procedure according to the invention, can be translated into an INR value. There is one row for each CT in seconds and one column for each temperature in degrees centigrade. If in practicing a PT procedure according to the invention, a temperature of 24°C and a clotting time of 45 seconds are measured, then the INR of the sample is 1.34. The section of a matrix shown covers the temperature range 19°C to 28°C. If, in connection with a PT test performed according to the invention, a temperature lower than 18°C is determined, then this is outside the conditions under which the test is intended, and no INR value is obtained. The temperature measured is thus used to qualify test conditions.

5 If the measured temperature is outside the permissible range, then the test conditions are not qualified and no test result will be generated.

10

	Temperature (°C)									
	19	20	21	22	23	24	25	26	27	28
CT: 29	0.46	0.50	0.55	0.60	0.66	0.71	0.78	0.84	0.91	0.98
30	0.48	0.53	0.58	0.63	0.69	0.75	0.82	0.89	0.96	1.03
31	0.51	0.55	0.60	0.66	0.72	0.79	0.86	0.93	1.00	1.08
32	0.53	0.58	0.63	0.69	0.76	0.82	0.90	0.97	1.05	1.12
33	0.55	0.60	0.66	0.72	0.79	0.86	0.94	1.01	1.09	1.17
34	0.58	0.63	0.69	0.75	0.82	0.90	0.98	1.06	1.14	1.22
35	0.60	0.66	0.72	0.79	0.86	0.94	1.02	1.10	1.19	1.27
36	0.63	0.69	0.75	0.82	0.89	0.97	1.06	1.15	1.23	1.32
37	0.65	0.71	0.78	0.85	0.93	1.01	1.10	1.19	1.28	1.37
38	0.68	0.74	0.81	0.89	0.97	1.05	1.14	1.24	1.33	1.42
39	0.71	0.77	0.84	0.92	1.00	1.09	1.19	1.28	1.38	1.47
40	0.73	0.80	0.87	0.96	1.04	1.13	1.23	1.33	1.43	1.53
41	0.76	0.83	0.91	0.99	1.08	1.17	1.27	1.38	1.48	1.58
42	0.79	0.86	0.94	1.03	1.12	1.22	1.32	1.42	1.53	1.63
43	0.81	0.89	0.97	1.06	1.16	1.26	1.36	1.47	1.58	1.68
44	0.84	0.92	1.01	1.10	1.20	1.30	1.41	1.52	1.63	1.74
45	0.87	0.95	1.04	1.13	1.23	1.34	1.45	1.57	1.68	1.79
46	0.90	0.98	1.07	1.17	1.27	1.38	1.50	1.62	1.73	1.85
47	0.93	1.01	1.11	1.21	1.31	1.43	1.55	1.67	1.79	1.90
48	0.96	1.04	1.14	1.24	1.36	1.47	1.59	1.72	1.84	1.96
49	0.99	1.08	1.18	1.28	1.40	1.52	1.64	1.77	1.89	2.02
50	1.02	1.11	1.21	1.32	1.44	1.56	1.69	1.82	1.95	2.07